**The Journal of Animal & Plant Sciences, 27(1): 2017, Page: 153-163**
**ISSN: 1018-7081**

# ENHANCEMENT OF PHYSIO-BIOCHEMICAL PARAMETERS OF WHEAT THROUGH EXOGENOUS APPLICATION OF SALICYLIC ACID UNDER DROUGHT STRESS

S. Noreen[1], K. Fatima[1], H. U. R. Athar[1], S. Ahmad[2] and K. Hussain[1]

[1]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Agronomy, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan
Corresponding author's email: sibgha_noreen@yahoo.com

## ABSTRACT

Water-stress is one of the major contributing factors, which limits the productivity of most of the crops plants under arid- and semi-arid environments. The proportionate reduction is associated with crop varieties, crop growth stage, and degree of severity of water stress under the given environments. The pot culture studies were conducted to quantify the efficacy of exogenously applied salicylic acid (SA) under drought environment on wheat crop as an amerliorative measure. The treatments consisted of three factors, (a) two watering regims (no water stress, water stress at 60 % moisture of the field capacity), (b) two levels of salicylic acid (0 mg $L^{-1}$, 200 mg $L^{-1}$), and (c) two wheat varieties (MH-97, S-24), arranged in a completely randomized design with four replications. The results showed that biological yield of variety MH-97 was higher by 42.71 % compared to S-24, while its quantity was reduced by 72.29 % due to drought condition. However, this reduction in biomass was mitigated by 20.1 % by spraying of 200 mg $L^{-1}$ salicylic acid. MH-97 was efficient in maintaining higher chlorophyll content by 3.2 % than S-24. The SPAD values of chlorophyll were higher by 46.0 % under drought stress compared to 42.11 % under fully irrigated crop, while, it was improved by 12.5 % by spray of SA. The spray of SA caused increase in total soluble proteins by 22.8 % over untreated check. The fully irrigated crop maintained 41.1 % higher free amino acids compared to drought stressed crop, while, quantity of amino acids were enhanced by 17.28 % by spray of salicyclic acid over untreated check. MH-97 contained an amount of 10.74 mg $K^+$ $g^{-1}$ compared to S-24 having 12.0 mg $K^+$ $g^{-1}$ nutrient. The water stressed crop maintained 15.43 % higher amount of $K^+$ compared to fully irrigated crop, and simultaneously improved by 15.34 % by spray of salicylic acid. Water stressed crop maintained 12.89 mg $Na^+g^{-1}$ compared to 11.26 mg $g^{-1}$ under fully irrigated condition. The spray of 200 mg $L^{-1}$ SA lowered the amount of $Na^+$ by 6.76 % compared to untreated check. The spray of SA improved 100-grain weight by 9.43 % over untreated check. MH-97 produced higher grain yield, 100-grain weight, number of spikes plant$^{-1}$, number of spiklets plant$^{-1}$, and number of tillers plant$^{-1}$. Therefore, the spray of SA at the rate of 200 mg $L^{-1}$ could rescue the wheat crop to withstand the vagaries of waterstress conditions.

**Keywords:** Salicylic acid, Drought stress, Wheat, Biological yield, Chlorophyll content, Free amino acids, $Na^+$ and $K^+$ constitutents, Grain yield and its components.

## INTRODUCTION

The water stress induced either by drought or salinity is the most damaging factor influencing the productivity to a greater extent across the globe (Davenport *et al*., 2003). The impact of water stress is compounded by depeletion of water in the rooting zone and increased atmospheric vapor pressure deficit (Ahanger *et al*., 2014). The reduction in productivity of the crop plants may vary from 50 to 73 % under limited water supplies (Berry *et al*., 2013). There are estimates that about 30-60% of total water applied to soil is lost through evaporation in the arid and semi-arid regions (Ashraf, 2010). Globally, the agricultural production areas are facing continuous decline in irrigation water supplies and also concurrent increase in salinization of soils due to application of underground brackish water (Cai *et al*., 2013). The frequent occurrence of drought condition is inevitable, but its ill-effects could be alleviated by adoption of certain short and long-term strategies (Ashraf, 2010). The plants being immobile in nature and thereby adapt certain diversed physiological, morphological, biochemical and molecular measures to cope with environmental stresses. The plants strive to mitigate the effects of water stress by over-production of scavenging phyto-hormones (Farooq *et al*., 2010b), activation of anti-oxidants battery system (Gill and Tuteja, 2010), upregulating osmotic adjustment (Ahmadi *et al*., 2001) and anatomical adaptations (Ashraf, 2010). The plants struggle a war to counter the deleterous effects of water stress conditions by adapting various mechanisms (Al-Hakimi, 2006). The strategy of winning a war by plants against water stress lies in the accumulation of greater quantities of organic solutes in the system for osmotic adjustment (Silva *et al*., 2010). Ahmad *et al*. (2015) found positive correlation among emergence index and emergence percentage of wheat crop both under normal as well as drought stress conditions. The drought tolerant wheat varieties maintained their cell membrance stability and integrity against water stress. The water stress affected cell membrance to disturb ion exchange and also causing imbalance in the composition of nutrients in the tissues. The increased production of osmolytes within the plant system protect the bio-chemicals against their deformation (Krasensky and Jonak, 2012). Moreover, they also work for scavanging free radicals to avoid damaging effects of reactive oxygen species (ROS) and deoxyribonucleic acid (DNA) (Rivas and Heitz, 2014). Hussain *et al*. (2015) reported that compatible solutes were efficacious in protecting cell organells from generation of free radicals under water stress. The stomata of sunflower crop were particularly closed due to spray of absciscic acid, which proved beneficial in protecting the plant tissues from dehydration. The organic osmolytes could be applied exogenously to induce the antioxidative defense system (Sliva *et al*., 2010). Various research studies have revealed that salicylic acid, an endogenous signal hormone is produced in a very low quantity and activates a number of physiological and biochemical processes in response to biotic and abiotic stressful conditions (Gunes *et al*., 2007). There are evidences that salicylic acid enhanced resistance to drought in maize crop (Hussain *et al*., 2010), wheat (Arfan *et al*., 2007), sunflower (Noreen *et al*., 2008), and cotton (Noreen *et al*., 2013) crop species. Salicylic acid has been found to be a potential osmolyte in enhancing stomatal conductance, photosynthatic capacity, transpiration efficiency, thermogeneses, osmoregulation, ion exclusion and synthesis of protein kinase (Noreen *et al*., 2013; Zahra *et al*., 2011).

Wheat (Triticum aestivum L.) is a staple food crop and is eaten by more than 50% of the popuplation in the world over. The productivity of wheat is highly sensitive to environmental vagaries, particularly, drought and temperature stresses, and resulting in yield reduction ranging from 40 to 50 % in most of the cases. The grain yield of wheat is affected due to adverse effects on cell development, physiological and biochemical attributes, metabolic processes, enzymatic dysfunctioning at different levels in the plant system (Ashraf, 2010). In the wake of increased risk at the mercy of climate change and its variability, there is clarion call for taking up practical and innovative measures to off-set the vagaries of environment. Jatoi *et al*. (2014) reported drought tolerance with regard to enhancement in yield and physiological traits could be improved substantially by utilizing heterosis technique. However, this requires heavy investment in capital goods and human resources. Alternatively, the foliar application of some of organic osmolytes could be employed in raising the tolerance level against the stressful conditions. The tolerance to drought condition could be enhanced by planting of drought tolerant crop varieties, adjusting crop husbandry practices in response to climatic conditions and/or adoption of shotgun measures such as foliar application of organic osmolytes (Deikman *et al*., 2012). The productivity of wheat crop was improved by 70% by application of salicylic acid (Kovacs *et al*., 2014). The accumulation of sodium and chloride was appreciably reduced by foliar spray of salicylic acid at the rate of 100 mg $L^{-1}$ in tomato crop under drought condition (Zahra *et al*., 2011). The efficacy of application of SA varies greatly in response to crop varieties, time of its application, stage of the crop growth, state of water stress, and environmental conditions. Therefore, the pot culture studies were undertaken to quantify the efficacy of salicylic acid as an amliorative measure to sustain wheat production under drought conditions.

## MATERIALS AND METHODS

The research study was undertaken to quantify the impact of salicylic acid on growth and development, biochemical and nutrient composition of wheat crop under drought condition at Bahauddin Zakariya University, Multan, Pakistan. The treatments consisted of three factors, [(a) two watering regimes (no water stress, water stress at 60 % moisture of the field capacity), (b) two levels of salicylic acid (0 mg $L^{-1}$, 200 mg $L^{-1}$), and (c) two wheat varieties (MH-97, S-24)], arranged in a completely randomized design with four replications. The seed was surface sterilized with 5.0 g $L^{-1}$ sodium hypochlorite for 5 minutes and dried before dibbling in plant pots. The plastic pots measuring diameter (24.5 x 28.0 cm) were filled with 10 kg of soil as a growth medium. The soil was ground and dried prior to filling in the pots. Twelve seeds were dibbled in each pot on 13th November, 2013. The seedlings were thinned to six number per pot after two weeks of germination. The salicylic acid (2-hydroxy benzoic acid) was dissolved in distilled water. The surfactant Tween-80 (polyoxyethylene sorbitol monolaurate) was used for preparation of spray solutions for fair absorption and penetration by the leaf tissues. The crop was exposed to water stress at day 35 (18th December, 2013) after sowing of crop. A quantity of 10 ml solution of salicylic acid was sprayed after 10 days of imposition of water stress (45 days after sowing of crop).

Data on various aspects were collected after 10 days after foliar application of salicylic acid (55 days after sowing of crop). The measurements on chlorophyll content were taken on the third fully expanded leaf from the top at 9:30 to 10:00 hours by using portable Chlorophyll Meter (Minolta Camera Co, Japan). After recording data, two plants were harvested along with the roots from each pot. The plant material was washed, blotted and divided into shoots and roots organs, and data collected on freshly produced biological yield. The total soluble proteins and free amino acids in the fresh plant material were determined according to methods of Bradford (1976) and Bates *et al*., (1973), respectively. The gathered biomass was placed in a forced – draft oven at 70 ºC at a constant and weighed to estimate biological yield. The remaining four plants were allowed to grow till reaching to their physiological maturity. Data on components of yield were recorded at maturity. The ionic constituents of $K^+$ and $Na^+$ were determined by employing the method of Ryan *et al.* (2001). Data were analyzed statistically by employing Statistical Software, COSTATv.6.3 (Cohort Software, Berkley, California) to determine significant variability among different factors. Means were compared by least significant difference test according to Steel et al.(1997).

## RESULTS

The chlorophyll content differed significantly ($p<0.001$) due to various treatments. Averaged across watering regimes and SA levels, the MH-97 maintained 3.2% higher chlorophyll content in comparison with S-24. Averaging the data for varietal and SA solution, the SPAD value of chlorophyll content was 46.0 under water stress conditions compared to 42.11 under fully irrigated crop. Averaged across varieties and watering regimes, the amount of chlorophyll content was improved by 12.5% through spraying of hormone in comparison with unsprayed crop drought environment. The stressed crop maintained 17.89% higher chlorophyll content over the untreated check (Table-2). Total soluble proteins differed significantly ($p<0.001$) due to various factors. Averaged across watering regimes and SA levels, MH-97 contained 1.46 mg $g^{-1}$ compared to 1.18 mg $g^{-1}$ of total soluble proteins maintained by S-24. Averaged across varieties and SA levels, the imposition of water stress resulted in the maintenance of higher total soluble proteins by 0.34 mg $g^{-1}$ content over fully irrigated crop. Averaged across varieties and watering regimes, the foliar application of hormone caused an increase in total soluble proteins by 22.88% higher over untreated check. Moreover, the crop sprayed with SA at the rate of 200 mg $L^{-1}$ contained 23.31% higher total content than untreated check (Table-2).

Total free amino acid differed significantly ($p<0.001$) due to varieties, watering regimes and salicylic acid levels. Averaged across watering regimes and SA levels, the variety MH-97 maintained 0.93 mg $g^{-1}$ compared to 0.84 mg $g^{-1}$ of total free amino acid by S-24. Averaged across varieties and SA levels, the water stressed crop contained 41.1% higher content of total free amino acid over the fully irrigated crop. Averaged across varieties and watering regimes, the crop sprayed with SA at the rate of 200 mg $L^{-1}$ maintained 17.28% higher free amino acid compared to unsprayed crop. The crop subjected to water stress contained 17.36% higher total amino acid over the untreated check under stressful condition (Table-2).

The $K^+$ concentration in shoot organ differed significantly due to varietal differences, watering regimes and SA levels. Averaged across watering regimes and SA level, MH-97 maintained $K^+$ concentration of 10.74 mg $g^{-1}$ compared to S-24 containing an amount of 12.0 mg $g^{-1}$. Averaged across varieties and SA level, water stressed crop maintained 15.43% higher concentration of $K^+$ in the shoot tissues compared to fully irrigated crop. Averaged across varieties and watering regimes, the foliar spray of SA caused an increase in $K^+$ concentration by 15.34% over untreated check. The exogenous application of hormone to water stressed crop resulted in an increase in $K^+$ by 17.63% over fully irrigated crop under stressful conditions (Table 3). The $K^+$ concentration in root tissues differed significantly ($p<0.001$) in response to varieties, watering regimes and SA levels. Averaged across watering regimes and SA levels, variety MH-97 maintained 7.66 mg $K^+$ $g^{-1}$ compared to 8.34 mg $K^+$ $g^{-1}$ by S-24. Averaged across varieties and SA levels, the crop subjected to water stress condition maintained 17.91% higher content of $K^+$ in their root tissues over the fully irrigated crop. Averaged across varieties and watering regimes, the application of SA caused an improvement in $K^+$ in root tissues by 19.78% over unsprayed treatment. Furthermore, the drought stressed crop maintained 19.11% higher content of $K^+$ in its root tissues over the untreated check under water deficit condition (Table-3).

The $Na^+$ concentration in root tissues varied to a significant level due to varieties, watering regimes and SA levels. Averaged across watering regimes and SA levels, MH-97 maintained 11.64 mg $Na^+$ $g^{-1}$ compared to 12.49 mg $Na^+$ $g^{-1}$ by S-24 in their shoot tissues. Averaged across variety and SA levels, the crop under water stress maintained 12.89 mg $Na^+$ $g^{-1}$ compared to 11.26 mg $Na^+$ $g^{-1}$ in their shoot tissues under fully irrigated crop. Averaged across varieties and watering regimes, the foliar spray of SA produced pronounced effects in lowering the amount of $Na^+$ in shoot tissues by 6.76% compared to untreated check. Furthermore, the foliar application of hormone reduced $Na^+$ in shoot tissues by 8.33% in drought stressed crop compared to fully irrigated crop. The $Na^+$ concentration in root tissues differed significantly in response to varieties, watering regimes and SA level. Averaged across watering regimes and SA level, MH-97 maintained lower concentration of 13.55 mg $Na^+$ $g^{-1}$ compared to 14.36 mg $Na^+$ $g^{-1}$ by variety S-24. Averaged across varieties and SA level, crop subjected to water stress maintained $Na^+$ at the level of 14.42 mg $g^{-1}$ compared to 13.49 mg under fully irrigated crop. Averaged across varieties and watering regimes, the ionic content of $Na^+$ was reduced 3.64% in root tissues in salicylic acid sprayed crop over the unsprayed crop. Moreover, drought stressed crop maintained lower concentration of Na$^+$ by 3.96% in root tissues compared to fully irrigated crop (Table 3).

The biological yield for shoots fresh and dry weight differed significantly ($p<0.001$) in response to varieties, watering regimes and salicylic acid levels. Averaged across watering regimes and salicylic acid levels, S-24 produced 42.71% higher quantities of biomass in comparison with MH-97. Averaged across varieties and SA levels, the biomass production was reduced by 70.2% due to imposition of water stress. On the other hand, biological yield was improved by 17.3% over unsprayed crop by exogenous application of 200 mg L$^{-1}$ salicylic acid. Furthermore, improvement in biological yield by 20.14% was witnessed by spray of SA under water stress compared to fully irrigated crop (Table-1). Averaged across other factors, S-24 produced 51.4% higher quantity of biomass in comparison with MH-97. The crop grown under normal irrigation management accumulated greater biomass by 38.69% over water stressed crop. However, enhancement in biomass was recorded by 32.5% by spraying the wheat crop with 200 mg L$^{-1}$ SA against the unsprayed crop. Moreover, spray of SA on water stress and crop also caused an increase in biological yield by 33.88% over fully irrigated crop (Table-1).

The quantities for roots fresh weight and dry weight differed statistically significant ($p<0.001$) due to various wheat varieties, watering regimes and salicylic acid levels treatments. Averaged across watering regimes and SA levels, the S-24 produced 0.74 and 0.52 g plant$^{-1}$ higher quantities of fresh and dry root biomass, respectively over the MH-97. The imposition of water stress caused reduction in the biological yield by maintaining 3.32 g plant$^{-1}$ and 0.38 g plant$^{-1}$ under no water stress and water stressed conditions, respectively. However, the reduction in biological yield was ameliorated by spraying the crop with salicylic acid solution. The increase in fresh and dry biological yield of roots was amounted by 47.25 and 112.5%, respectively over untreated check under drought environments (Table-1).

The components of wheat grain yield differed significantly in response to varieties, watering regimes and salicylic acid levels (Table 4). Averaged across watering regimes and salicylic acid levels, MH-97 had 6.48% heavier 100-grain compared to S-24. Averaged across varieties and salicylic acid, 100-grain weight was observed heavier by 2% under no water stress compared to water stressed crop. The gain in grain wheat witnessed by 9.43% in crop sprayed with salicylic acid over the unsprayed crop. Averaged across watering regimes and salicylic acid, MH-97 produced higher grain yield by 24.53 % over variety S-24. Averaged across varieties and salicylic acid, grain yield was higher by 53.61 % under no water stress compared to water stress crop.

The enhancement in grain yield was witnessed by 2.3 % in sprayed crop in comparison with unsprayed crops. Averaged across watering regimes and SA levels, MH-97 produced 5.61 % higher number of spikes plant$^{-1}$ over S-24. Averaged across varieties and SA levels, crop fully irrigated produced 2.98 percent higher number of spikes plant$^{-1}$over drought stressed crop. Averaged across varieties and watering regimes, the spray of crop with 200 mg L$^{-1}$ SA caused an increase by 15.85% higher number of spikes plant$^{-1}$over untreated check. The MH-97 produced 1.39% higher number of spiklets plant$^{-1}$ over S-24. The fully irrigated crop produced 34.89% higher number of spikes plant$^{-1}$ over drought stressed crop. Furthermore, spray of SA at the rate of 200 mg L$^{-1}$ produced 14.84% higher number of spikelet plant$^{-1}$ over untreated check. Averaged across watering regimes and SA levels, the numbers of tillers plant$^{-1}$ were higher by 1.8 % in MH-97 over the S-24. The fully irrigated crop produced higher number of tillers plant$^{-1}$ by 3.6 % over the drought stressed crop. The number of tiller plant$^{-1}$ were increased by 18.5% in crop sprayed with salicylic acid compared to untreated check.

**Table 1. Fresh and dry weights of shoots and roots of wheat varieties in response to foliar spray of salicylic acid grown under drought conditions (Mean ±S.E, *n*=5).**

| **Treatments** | | **Shoot fresh weight (g plant$^{-1}$)** | | **Shoot dry weight (g plant$^{-1}$)** | | **Root fresh weight (g plant$^{-1}$)** | | **Root dry weight (g plant$^{-1}$)** | |
|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **Watering regime** | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | |
| | | **0** | **200** | **0** | **200** | **0** | **200** | **0** | **200** |
| MH-97 | No water stress | $11.9\pm0.9^a$ | $14.5\pm0.9^a$ | $1.55\pm0.08^a$ | $1.79\pm0.3^a$ | $1.01\pm0.15^a$ | $1.55\pm0.2^a$ | $0.23\pm0.03^a$ | $0.26\pm0.04^a$ |
| | Water stress | $4.42\pm1.0^b$ | $5.29\pm0.7^b$ | $0.97\pm0.04^a$ | $1.62\pm0.3^a$ | $0.32\pm0.07^b$ | $0.66\pm0.2^b$ | $0.13\pm0.01^a$ | $0.31\pm0.05^a$ |
| S-24 | No water stress | $18.8\pm0.6^a$ | $21.7\pm0.7^a$ | $2.44\pm0.23^a$ | $3.44\pm0.1^a$ | $1.55\pm0.04^a$ | $1.46\pm0.1^a$ | $0.39\pm0.03^a$ | $0.50\pm0.05^a$ |
| | Water stress | $4.12\pm0.5^b$ | $4.97\pm0.8^b$ | $1.63\pm0.17^b$ | $1.33\pm0.2^b$ | $0.59\pm0.63^b$ | $0.68\pm0.1^b$ | $0.19\pm0.03^a$ | $0.37\pm0.03^a$ |
| LSD at 5% | | 4.096 | | 0.974 | | 0.564 | | 0.182 | |

**Table 2. Total chlorophyll content, total soluble proteins and total free amino acid in wheat varieties in response to foliar spray of salicylic acid grown under drought conditions (Mean ±S.E, *n*=5).**

| **Treatments** | | **Total chlorophyll (SPAD values)** | | **Total soluble proteins (mg g$^{-1}$)** | | **Total free amino acids (mg g$^{-1}$)** | |
|---|---|---|---|---|---|---|---|
| **Variety** | **Watering regime** | **SA level, mg L$^{-1}$** | | **SA level, mgL$^{-1}$** | | **SA level, mg L$^{-1}$** | |
| | | **0** | **200** | **0** | **200** | **0** | **200** |
| MH-97 | No water stress | 40.73±0.76$^{a}$ | 43.21±1.50$^{a}$ | 1.17±0.29$^{a}$ | 1.41±0.24$^{a}$ | 0.74±0.08$^{a}$ | 0.83±0.05$^{a}$ |
| | Water stress | 44.65±3.18$^{a}$ | 50.40±1.19$^{a}$ | 1.649±0.25$^{a}$ | 1.75±0.25$^{a}$ | 1.00±0.60$^{b}$ | 1.13±0.14$^{b}$ |
| S-24 | No water stress | 40.39±1.17$^{a}$ | 44.13±1.09$^{a}$ | 0.89±0.06$^{a}$ | 1.11±0.23$^{a}$ | 0.60±0.07$^{a}$ | 0.74±0.14$^{a}$ |
| | Water stress | 40.07±1.66$^{a}$ | 48.87±0.73$^{a}$ | 1.17±0.19$^{b}$ | 1.53±0.53$^{b}$ | 0.90±0.07$^{a}$ | 1.10±0.06$^{b}$ |
| LSD at 5% | | 8.214 | | 1.519 | | 0.509 | |

**Table 3. Ionic constituents ($K^+$, $Na^+$) in shoot and root tissues of wheat varieties in response to foliar spray of salicylic acid grown under drought conditions (Mean ± S.E, *n*=5).**

| **Treatments** | | **Shoot $K^+$ (mg g$^{-1}$)** | | **Root $K^+$ (mg g$^{-1}$)** | | **Shoot $Na^+$ (mg g$^{-1}$)** | | **Root $Na^+$ (mg g$^{-1}$)** | |
|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **Watering Regime** | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | | **SA level, mg L$^{-1}$** | |
| | | **0** | **200** | **0** | **200** | **0** | **200** | **0** | **200** |
| MH-97 | No water stress | 9.73±1.9 $^{a}$ | 10.21±1.3$^{a}$ | 6.19±1.4$^{a}$ | 7.93±1.8$^{a}$ | 11.1 ±0.7 $^{a}$ | 10.7±0.4$^{a}$ | 13.17±1.9$^{a}$ | 12.75±1.67$^{a}$ |
| | Water stress | 10.22±1.9$^{a}$ | 12.78±1.9$^{b}$ | 7.61±2.5$^{a}$ | 8.89±3.5$^{b}$ | 12.79±1.1$^{a}$ | 12.00±0.4$^{a}$ | 14.29±4.2$^{b}$ | 14.00±5.3$^{b}$ |
| S-24 | No water stress | 10.11±1.1$^{a}$ | 12.19±3.1$^{a}$ | 7.11±1.5$^{a}$ | 8.13±1.8$^{a}$ | 12.22±1.4$^{a}$ | 11.00±1.1$^{a}$ | 14.25±5.7$^{a}$ | 13.79±1.6 $^{a}$ |
| | Water stress | 12.19±1.9$^{a}$ | 13.57±1.4$^{a}$ | 8.19±1.9$^{a}$ | 9.93±2.3$^{a}$ | 13.75±2.1$^{a}$ | 13.00±1.3$^{a}$ | 15.11±7.5 $^{a}$ | 14.29±4.3 $^{b}$ |
| LSD at 5 % | | 10.473 | | 12.57 | | 8.933 | | 106.98 | |

**Table 4. Wheat grain yield and its components of wheat varieties in response to foliar spray of salicylic acid grown under drought conditions (Mean ± S.E, *n*=5).**

| **Treatments** | | **100-Grain weight (g)** | | **Total grain weight (g plant$^{-1}$)** | | **No. of spikes plant$^{-1}$** | | **No. of spikelets plant$^{-1}$** | | **No. of tillers plant$^{-1}$** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | Watering regime | SA level, mg L$^{-1}$ | | SA level, mg L$^{-1}$ | | SA level, mg L$^{-1}$ | | SA level, mg L$^{-1}$ | | SA level, mg L$^{-1}$ | |
| | | 0 | 200 | 0 | 200 | 0 | 200 | 0 | 200 | 0 | 200 |
| MH-97 | No water stress | 3.21+ 0.17 | 3.48+ 0.13 | 6.59± 0.34$^{a}$ | 7.15± 0.41$^{a}$ | 3.13± 0.35$^{a}$ | 3.99± 0.15$^{a}$ | 60.7± 5.5$^{a}$ | 65.5± 5.8a | 4.20.4$^{a}$ | 4.9± 0.3$^{a}$ |
| | Water stress | 0.16+ 0.01 | 1.28+ 0.111 | 3.67± 0.14$^{b}$ | 3.91± 0.53$^{b}$ | 3.5± 0.27$^{a}$ | 4.15± 0.30$^{a}$ | 48.5± 3.8$^{b}$ | 58.6± 6.0$^{a}$ | 4.8± 0.4$^{a}$ | 5.6± 0.5$^{a}$ |
| S-24 | No water stress | 3.11+ 0.05 | 3.37+ 0.052 | 5.42± 0.50$^{a}$ | 5.79± 0.55$^{a}$ | 3.88± 0.38$^{a}$ | 4.2± 0.31$^{a}$ | 65.1± 2.9$^{a}$ | 73.6± 5.6$^{a}$ | 4.7± 0.5$^{a}$ | 5.6± 0.7$^{a}$ |
| | Water stress | 0.89+ 0.08 | 1.38+ 0.15 | 3.43± 0.37$^{b}$ | 3.21± 0.39$^{b}$ | 4.2± 0.38$^{a}$ | 3.69± 0.38$^{a}$ | 4.0± 5.5$^{a}$ | 50.0± 5.7$^{a}$ | 4.0± 0.4$^{b}$ | 4.9± 0.4$^{a}$ |
| LSD at 5% | | 0.45 | | 3.437 | | 1.4966 | | 23.77 | | 2.067 | |

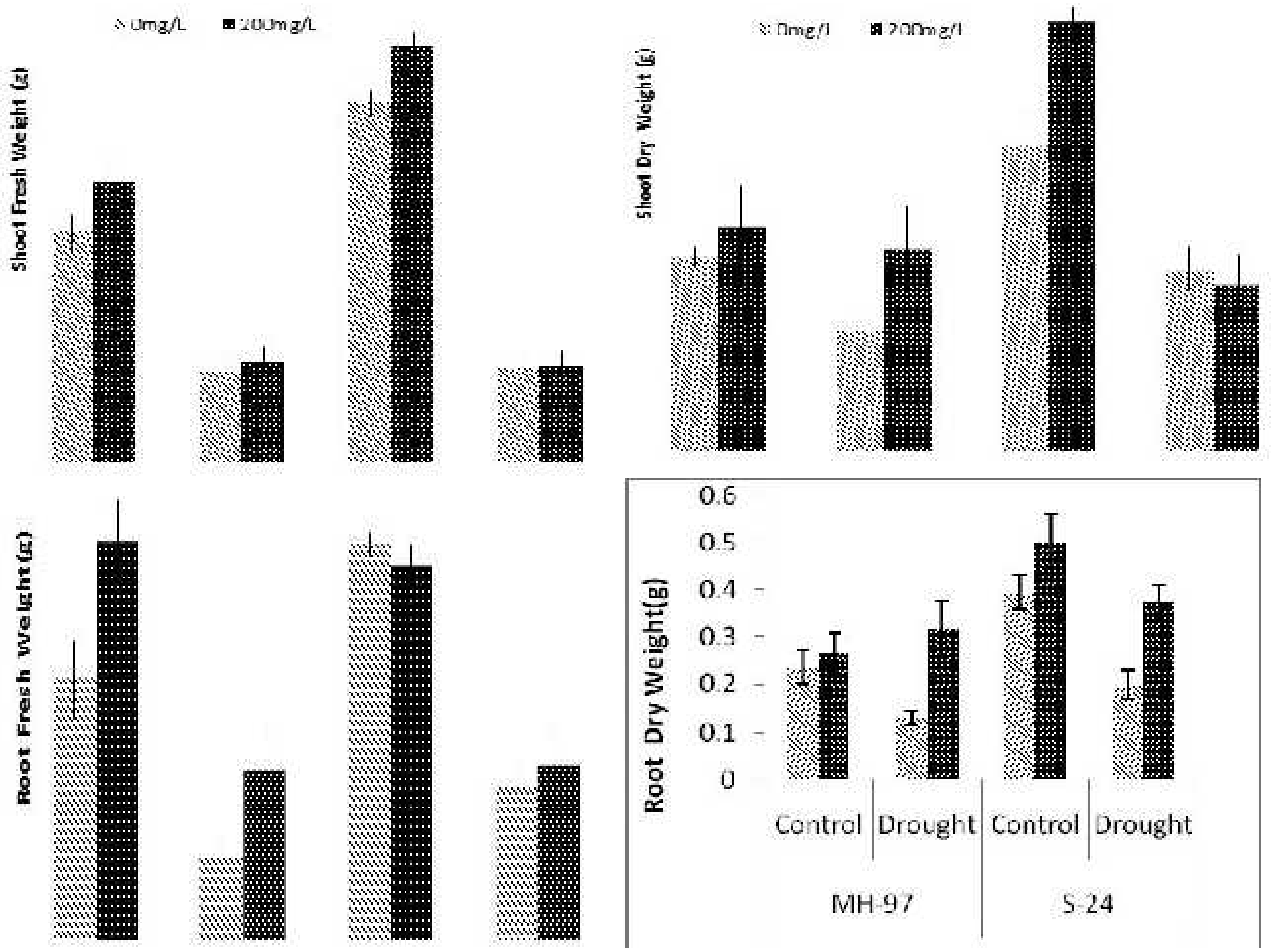
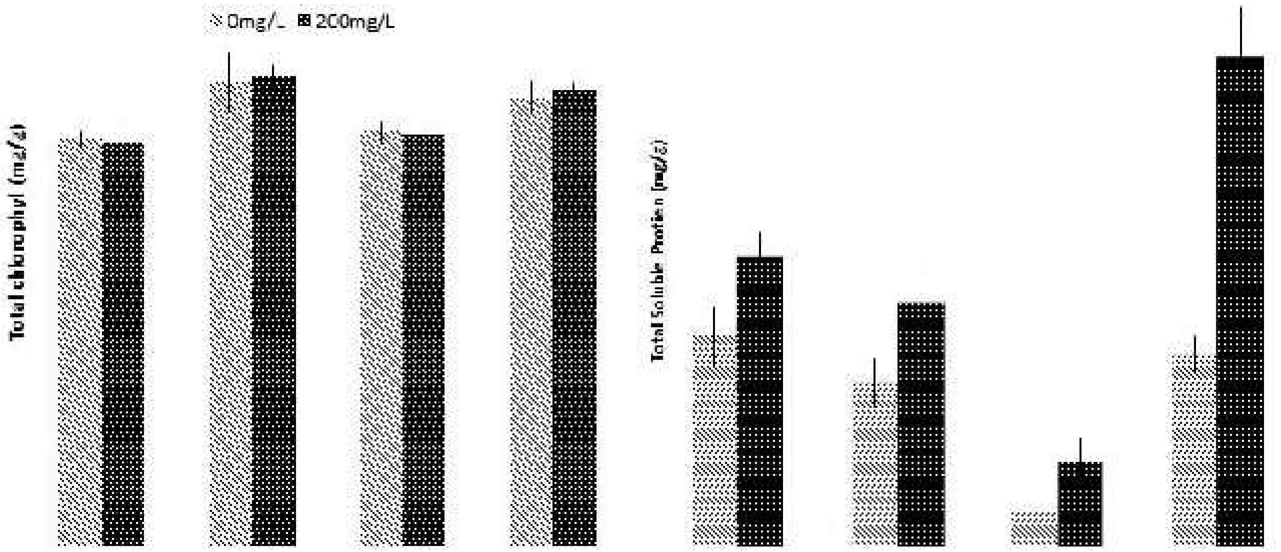


**Fig. 1. Fresh and dry weights of shoots and roots of wheat varieties in response to salicylic acid grown under drought condition.**



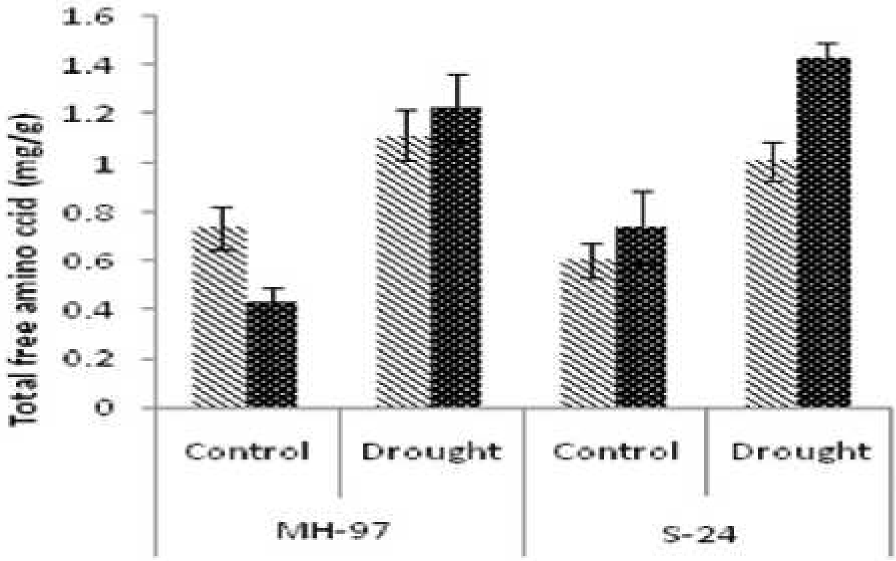
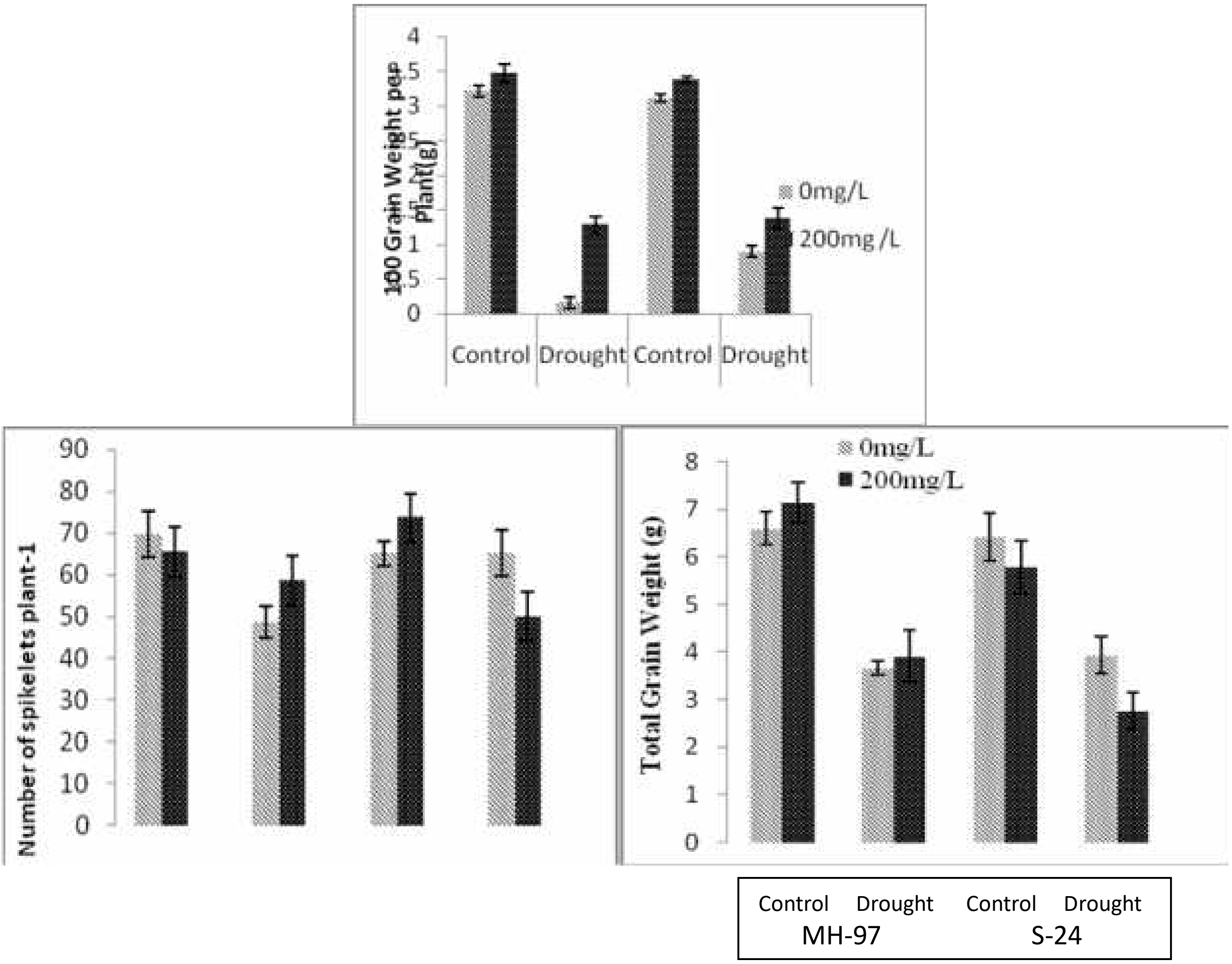


**Fig. 2. Total chlorophyll content, total soluble proteins and total free amino acids of wheat varieties in response to salicylic acid grown under drought condition.**



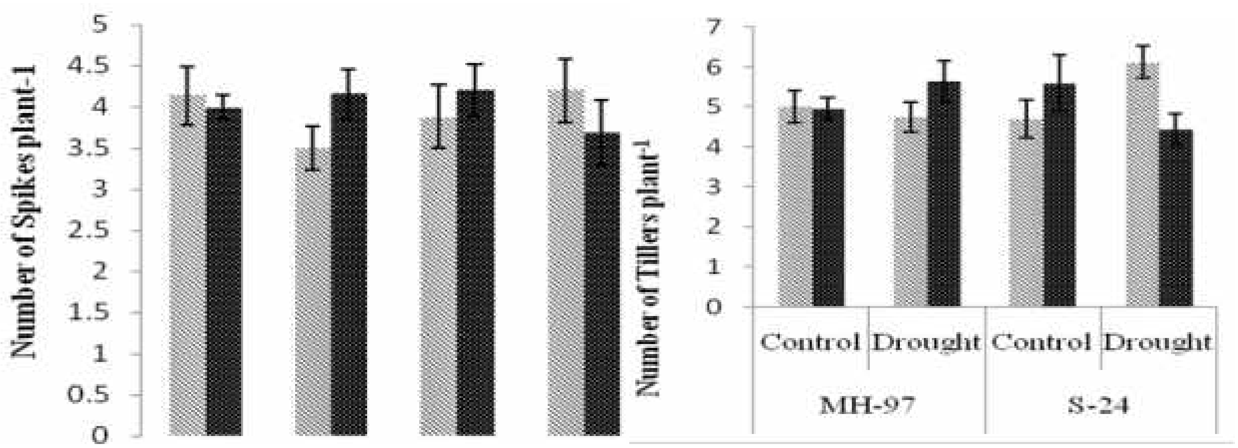


**Fig. 3: 100-grain weight, total grain weight, no. of spikes, spikelets and tillers of wheat varieties in response to salicylic acid grown under drought conditions.**

## DISCUSSION

Salicylic acid is an endogenous hormone, which regulates a number of physiological and biochemical processes, i.e. progressive growth, seed development, productivity per unit land area flower production, reducing the generation of reactive oxygen species (ROS) and up-regulating the defense system to maintain the osmotic adjustment during the stressful environment (Hasanuzzaman *et al.,* 2014). Moreover, the modulating effect exerted by external application of salicylic acid enhanced the photosynthetic capacity and stomatal conductance (Noreen and Ashraf, 2008). The quantity and proportionate amounts of nutrient ions were also altered as induced by addition of salicylic acid under stressful condition (Zahra *et al.,* 2011).Various researchers have reported that oxidative stress caused by generation of reactive oxidative species (ROS) under drought stress could be quenched by increasing the quantum of salicylic acid in the plant system (Eyidogan *et al.,* 2012). In a recent study, Hasanuzzaman *et al.* (2014) also reported that salicylic acid induced anti-oxidative system resulted in ameliorating the adverse effect of environmental stresses.

The results of the present study demonstrated that growth, development and a number of physiological functions including photosynthetic activity were impacted to a greater extent in response to drought stress. The reduction in photosynthetic capacity resulted in decreased production of biological yield and ultimately led to the loss of yield (Ashraf *et al*., 2012; Chaves *et al*., 2011). Various researchers (Farooq *et al*., 2013; Zhang *et al*., 1998) also found a close correlation between photosynthetic capacity and progressive plant growth exogenous application of salicylic acid alleviated the adverse effects of drought stress and enhanced the growth and development of both varieties of wheat crop.

The results corroborates with those of (Misra *et al*., 2014; Kong *et al*., 2014, Hasanuzzaman *et al*., 2014) that raising the level of salicylic acid either by foliar spray and/or due to over-production of organic osmolytes in the plant system would reduce the damaging effects of drought stress on growth and development of various crop species. The chlorophyll content in both wheat varieties was greatly limited in response to water stress environment. The process of photosynthesis is regulated by chlorophyll ‘a’ and ‘b’ (Kalaji *et al*., 2011). In analogous to this study, the reduction in chlorophyll content due to water stress has also been reported in other crops; wheat (Stiven and Sanaratna, 2006), soybean (Zhang *et al*., 2004) and maize (Farooq *et al*., 2009). However, improvement in chlorophyll content has also been reported by foliar spray of salicylic acid on various crops, soybean (Kalaji *et al*., 2011) and cotton (Noreen *et al*., 2012) under both no-water stress and water stress environments.

The prevalence of drought condition disturbed the ionic balance and thereby impacting the physiological processes of the plant (Hasanuzzaman *et al*., 2014). The drought stress depressed the absorption of $K^+$ ion and enhanced the uptake of $Na^+$ ion in both wheat varieties. On the other hands, the foliar application of salicylic acid favoured the uptake of $K^+$ ion at the cost of $Na^+$ in the plant system. Similar results are reported by Hussain *et al*. (2010) that increase in drought stress enhanced the uptake of $Na^+$ ion and conversely, the absorption of $K^+$ ion by maize and barely crop plants. Singh and Usha (2003) also found that treating wheat seed with salicylic acid solution enhanced the level of tolerance in response to drought stress. The plant accumulated the greater quantity $K^+$ ion in their tissues, while the absorption of $Na^+$ was reduced markedly. Thereby, the greater translocation of $K^+$ from roots to the upper parts of the plants caused improvement in the growth and development of wheat crop.

The results further indicated that S-24 maintained high quantity of soluble protein compared to

MH-97 in response to water stress. Hayat *et al*. (2007) reported that activity of synthesis of protein in plants was enhanced greatly under stressful condition. Different researchers (Ahmad *et al*., 2010; Ahmad *et al*., 2013) also reported that quantity of soluble protein increased with the increasing level of salinity in barley and chlorella plants. However, Chandra *et al*. (2007) found that drought stress caused reduction in soluble protein contents in sorghum and tomato crops. The reason being that reduction in protein is due to breakdown of protein into amino acids under water stress condition. The findings of our study are correlated with those of (Farooq *et al*; 2010a; Farooq *et al*., 2013) that quantum of soluble protein increased in rice and wheat crop plants under stressful condition. Data from this study also indicated that total free amino acids increased in both wheat varieties, when they were grown under drought conditions. Various researchers also reported that application of salicylic acid produced stimulating effects in increasing the amount of total soluble protein and total free amino acids in maize crop in response to drought stress (Azooz *et al*., 2013). These results agree with those of Chandra *et al*., (2007) that quantity of free amino acids were increased in most of crop plants in response to drought stress.

The biological yield gathered from both wheat varieties decreased in response to drought stress in comparison with no water stress. The progressive improvement in the biological yield is resultant of sustainable photosynthetic capacity and/or over-production of scavenging phyto hormones (Farooq *et al*., 2010b). The existence of negative association between photosynthetic capacity and biomass production has also been reported by Loutfy *et al*. (2012). The improvement of biological yield due to spray of salicylic acid on various crop plants under drought stress conditions have been reported (Yang and Lu, 2005; Noreen *et al*., 2012). The results of the study are in agreement with those of Abdallah and Khoshibon (2007) and Noreen *et al*. (2013). The improvement in grain yield and its components have been an outcome of sustained photosynthetic capacity of wheat plants due to greater accumulation of organic osmolytes. The presence of increase amount of salicylic acid resulted in improvement in the tolerance to drought condition. These results agree with those of Hussain *et al*., 2010 and Noreen *et al*. 2013. It is concluded from this study that various physiological parameters of wheat crop could be enhanced and/or sustained by foliar spray of 200 mg litre$^{-1}$ salicylic acid under drought stress environment.

## REFERENCES

Abdalla, M. M. and N. H. El-Khoshiban (2007). The influence of water stress on growth, relative water content, photosynthetic pigments, some metabolic and hormonal contents of two *Triticium aestivum* L. cultivars. Appl. Sci. 3: 2062-2074.

Ahanger, M.A., S.R. Tyagi, M.R. Wani, and P. Ahmad (2014). Drought tolerance: Role of organic osmolytes, growth regulators, and mineral nutrients. Physiological Mechanism and Adaptative Strategies in Plants under Changing Environment. Springer, pp 25-55.

Ahmad, I., S.H.A. Basra, I. Afzal, M. Farooq, and A. Wahid (2013). Growth improvement in spring maize through exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide. Int. J. Agric. Biol. 15: 95-100.

Ahmad, I., I. Khaliq, N. Mahmood, and N. Khan (2015). Morphological and physiological criteria for drought tolerance at seedling stage in wheat. The J. Anim. Plant Sci. 25(4): 1041-1048.

Ahmad, P., C.A. Jaleel, M.A. Salem, G. Nabi, and S. Sharma (2010). Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit. Rev. Biotechnol. 30: 161-175.

Ahmadi, A., and D.A. Baker (2001). The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. Plant Growth Regul. 35: 81-91.

Al-Hakimi, A.M.A. (2006). Counteraction of drought stress on soybean plants by seed soaking in salicylic acid. Int. J. Bot. 2: 421-426.

Arfan, M., H.R. Athar and M. Ashraf (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in differently adapted spring wheat cultivated under salt stress? Plant Physiol. 6: 685-694.

Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. Biotchnol. Adv. 28: 169-183.

Ashraf, M., M.S.A. Ahmad, M. Ozturk, and A. Aksoy (2012). Crop improvement through different means: Challenges and prospects. Crop. Prod. Agric. Improv. 1-15.

Azooz, M.M., A.M. Alzahrani, and M.M. Youssef (2013). The potential role of seed priming with ascorbic acid and nicotinamide and their interactions to enhance salt tolerance in broad bean (*Vicia faba* L.). Aust. J. Crop Sci. 7: 2091.

Bates, L.S., R.P. Waldren, and I.D. Tear (1973). Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205-207.

Berry, P., J. Ramirez-villegas, and H. Branseley (2013). Regional impacts of climate change on agriculture and the role of adaptation. Plant Genetic Resource and Climate Change, 4:78.

Bradford, M. M. (1976). A rapid and sensitive method for the quantification of microgram of protein utilizing the principle of dye binding. Ana. Biochem. 72: 248-254.

Cai, X., D. Molden, M. Mainuddin, B. Sharma, A. Mohyddin, and P. Karini (2013). Producing more food with less water in the changing world: Assessment of water productivity in 10 major river basins. Water Intl. 36: 421-62.

Chandra, A., A. Anand, and A. Dubey (2007). Effect of salicylic acid on morphological and biochemical attributes in cowpea. J. Environ. Biol. 28: 193-196.

Chaves, M., J. M. Costa and N. J. M. Saibo (2011). Recent advances in photosynthesis under drought and salinity. Adv. Bot. Res. 57: 49-104.

Davenport, S.B., S.M. Gallego, M.P. Benavides and M.L. Tomaro (2003). Behaviour of anti-oxidant defence system in the adaptive response to salt stress in *Helianthus annuus* L. Cell Plant Growth Regul. 40: 81-88.

Deikman, J., M. Petracek, and J. E. Heard (2012). Drought tolerance through biotechnology: Improving translocation from the laboratory to farmers' fields. Current Opinion. Biotech. 23: 243-250.

Eyidogan, F., M.T.Oz., M. Yucel, and H.A. Oktem (2012). Signal transduction of phytohormones under abiotic stresses. In: Khan, N.A., R. Nazar, N. Iqbal, N.A. Anjum (eds). Phytohormones and Abiotic Stresses Tolerance in Plants. Springer, pp 1-48.

Farooq, M. A., D.J. Lee. Wahid, S. Cheema, and T. Aziz (2010 b). Drought Stress: Comparative time course action of the foliar applied glycine betaine, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. J. Agron Crop Sci. 196: 336-345.

Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra (2009). Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185-212

Farooq, M., M. Irfan, T. Aziz, I. Ahmad, and S. Cheema (2013). Seed priming with ascorbic acid improves drought resistance of wheat. J. Agron.Crop Sci. 199: 12-22.

Farooq, M., N. Kobayashi, O. Ito, A. Wahid, and R. Serraj (2010a). Broader leaves result in better performance of indica rice under drought stress. J. Plant Physiol. 167: 1066-1075.

Foyer, C.H., G. Noctor (2013). Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Phyiol. Plant, 119: 355-364.

Gill, S.S., and N. Tutej (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48: 909-930.

Gunes, A., AInal, M. Alpaslan, F. Eraslan, E.Guneri Bagci, and N. Cicek (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J. Plant Physiol. 164: 728-736.

Hasanuzzaman, M., M.M. Alam, K. Nahar, Jubayer-Al-Mahmud, K.U. Ahmad, and M. Fujita (2014). Exogenous salicylic acid alleviates salt-stress oxidative damage in *Brassica napes* by enhancing the anti oxidant defense and glyoxalase systems. Aust. J. Crop. Sci. 8: 631-648.

Hayat, S., B. Ali and A. Ahmad (2007). Salicylic acid: biosynthesis, metabolism and physiological role in plant. In: Salicylic Acid A Plant Hormone. Hayat, S. and A. Ahmad (Eds.), Springer, Drodrecht, pp: 1-14.

Hussain, K., K. Nawaz, and A. Majeed (2010). Alleviation of salinity effects by exogenous application of salicylic acid in pearl millet (*Pennisetum glaucum* L.) seedlings. Afr. Biotechnol. 9: 8602-8607.

Hussain, S., M. F. Saleem, J. Iqbal, M. Ibrahim, M. Ahmad, S. M. Nadeem, A. Ali and S. Atta (2015). Abscic acid mediated biochemical changes in sunflower (Helianthus annuus L.) grown under drought and well-watered field conditions. The J. Amim. Plant Sci. 25(2): 406-414.

Jatoi, W. A., M. J. Baloch, N.U. Khan, M. Munir, A. A. Khakwani, N.F. Vessar, S.A. Panwar and S. Gul (2014). Heterosis for yield and physiological traits in wheat under water stress conditions. The J. Anim. Plant Sci. 24(1): 252-261.

Kalaji, M. H., K. Bosa, J. Kościelniak and Z. Hossain (2011). Chlorophyll "*a*" fluorescence – A useful tool for the early detection of temperature stress in spring barley (*Hordeum vulgare* L.). OMICS: African J. of Integ. Biol. 15: 925–934.

Kong, J., Y. Dong, L. Xu, S. Liu, and X. Bai (2014). Role of exogenous nitric oxide in alleviating iron deficiency-induced peanut chlorosis on calcareous soil. J. Plant Inter. 9: 450-459.

Kovacs, V., O.K. Gondor, G. Szalai (2014). Synthesis and role of salicylic acid in wheat varieties with different levels of cadmium tolerance. J. Hazard Material. 280: 12-19.

Krasensky, J., and C. Jonak (2012). Drought, salt, and temperature stress induced metabolic re-arrangements and regulatory networks. J. Expt. Bot. 63: 1593-1608.

Loutfy, N., M.A. El-Tayeb, A.M. Hassanen, M.F. Moustafa, Y. Sakuma, and M. Inouhe (2012). Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum* L.). J. Plant Res. 125: 173-184.

Misra, N., R. Misra, A. Mariam, K. Yusuf, and L. Yusuf. 2014. Salicylic acid alters antioxidant and phenolics metabolism in *Catharanthus roseus* grown under salinity stress. African J.Trad, Comple. Alter. Medi. 11: 118-125.

Noreen, S., H.R. Athar and M. Ashraf (2013). Interactive effects of watering regimes and exogenously applied osmoprotectant on earliness indices and leaf area index in cotton (*Gossypium hirsutum* L.) crop. Pakistan J. Bot. 45: 1873-1881.

Noreen, S., M. Ashraf and N.A. Akram (2012). Does exogenous application of salicylic acid improves growth and some key physiological attributes in sunflower plants subjected to salt stress? J. Appl. Bot. Food Qual. 84: 169-180.

Noreen. S, and M. Ashraf (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. Pakistan J. Bot., 40,1657.

Rivas, S., T. Heitz (2014). Phospholipase A in plant immunity. *Phospholipases* in Plant Signaling , Springer, pp. 183-205.

Ryan, J., G. Estefan, and A. Rashid (2001). Soil Analysis Laboratory Manual. 2nd ed. Intl. Center for Agric. Res. In Dry Areas (ICARDA). Syria. 172p.

Silva, E.N., S.L. Ferreira-Silva, R.A. Viegas, and J.A.G. Silveira (2010). The role of organic and inorganic solutes in the osmotic adjustment of drought stressed *Jatropha cureas* plants. Environ. Expt. Bot. 69: 279-285.

Singh, B. and K. Usha (2003). Salicylic acid induced physiological and biochemical changes in wheat seedling sunder water stress. Plant Growth Regul. 39:P 137-141.

Steel, R. G. D., and J. H. Torrie and D. A. Dickey (1997). Principles and Procedures of Statistics: A Biometrical Approach 3rd Edition. McGrew Hill Book Co., New York. pp 377-398.

Stiven, G. and T. Sanaratna. 2006. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regul. 39: 137-141.

Yang, X., and C. Lu (2005). Photosynthesis is improved by exogenous glucinebetaine in salt-stressed maize plants. Physiol. Plant. 124: 343-352.

Zahra, S., B. Amin, V.S.M. Ali, Y. Ali, and Y. Mehdi (2011). The salicylic acid effect on the tomato (*Lycopersicum esculentum* Mill), sugar, protein, and proline contents under salinity stress (NaCl). J. Biophysics Stru. Biol. 2: 35-41.

Zhang, M., L. Duan, Z, Zhai, J. Li, X. Tian, B. Wang, Z. He, and Z. Li (2004). Effect of plant growth regulators on water deficit induced yield loss in soybean. Proc. 4th Int. Crop Sci. Cong. Brisbane, Australia.

Zhang, S.G., J.Y. Gao, and J.Z. Song (1998). The effects of salicylic acid and aspirin on the $Na^+$, $K^+$ and $Cl^-$ contents and distribution in wheat seedlings under salinity stress. Beij. Agri. Sci. 16: 1-15.